

Human colostrum: a natural source of probiotics?

Colostro humano: fonte natural de probióticos?

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Introduction

The first scientific studies on probiotics date back to the beginning of the century with the work of Metchnikoff at the Pasteur Institute. This investigator postulated that hosts could benefit from fermented milk in the sense that it antagonizes bacteria that affect the intestines(1-3). The initial hypothesis posited that bacterial strains with more efficient mucosal adherence would be more beneficial for their hosts(2).

Recent scientific data have underscored the importance of the intestinal flora for human health. The benefits of probiotics include antagonizing pathogenic agents, promoting a defense barrier against the microbiota, and modulating immune responses(2,4-6).

For several years, researchers have tried to isolate, identify, and characterize the microorganisms found in human intestines. However, the complete assessment of this microbiota is an extremely difficult process(7).

One of the current definitions of probiotics is that they are viable microorganisms that have a beneficial effect in the prevention and treatment of specific pathologic conditions by improving microbial balance of the gastrointestinal tract(1,2).

At birth, the immature human gut is germ-free, but bacterial colonization begins during delivery. Soon, several microorganisms are introduced into the gut flora with the diet(8). Under normal conditions, the maternal intestine microbiota will be the main source of bacteria that colonize the gastrointestinal tract of the newborn(9).

Exclusive breastfeeding is recognized as the best means to protect newborn infants from infectious diseases; in part, this protection is probably due to the influence of maternal milk on the composition of intestine microbiota of newborns(10,11). In developing countries, where there is greater probability of newborn infants being exposed to several bacteria from birth, breastfeeding is considered extremely important. That is because maternal milk protects infants from several infections such as diarrhea, septicemia, respiratory tract infections, and so on, thus reducing infant mortality rates(8).

Breast-fed infants present fecal flora different from that of formula-fed infants. The earlier have a preferred intestine microbiota in which bifidobacteria and lactobacillus predominate over potentially harmful bacteria, whereas in the latter, coliforms, enterococci, and bacteroides predominate(2).

The colonization of the intestines by different bacterial strains is regulated by the intestine medium, which changes according to colonization by new groups of bacteria(8). It is suggested that the size of bacterial population is rigorously controlled by the competition for

nutrients and space(8). Thus, potential pathogenic agents such as the *E. coli* and other enterobacteria are suppressed, and so is the colonization by new bacteria. This function of intestine microbiota is called "resistance to colonization"(12). This microbiota operates as an important component of the mucosal immune response(5).

The response to intestine microbiota results in formation of serum antibodies against several bacterial structures of the intestine, and is extended to other mucosas and to exocrine glands such as the salivary and mammary glands during lactation(2,5).

After reacting to bacterial components at specific sites called Peyer's patches, the lymphocytes migrate to other parts of the intestinal wall spreading the immune response throughout the intestines. These lymphocytes are translocated, by means of complex mechanisms, to the maternal milk and, through breastfeeding, will later act on the intestine bacteria of newborn infants protecting babies from infections(13).

The immune response can help to control intestinal bacteria, limit their translocation, and reduce infection risks. With time, intestinal microbiota can induce immunologic tolerance, resulting in reduction of the ability to react to some of its components. When potentially pathogenic microbial agents are delivered to the intestine, the organism of the host will produce a new immune response(14,15).

Bacteria constantly reinforce the lines of defense of the intestine through mechanisms of immune exclusion, elimination of immune character, and immune regulation that allow for a dynamic commensal relationship between human beings and microorganisms(16).

The Brazilian Ministry of Health defines the human colostrum as the secretion by the mammary glands of the mother until the seventh day postpartum(17). In the donation of colostrum to Human Milk Banks, it is important to consider that the magnitude of secondary contaminating agents incorporated into the product during collection can have a decisive effect on its final quality. Thus, the presence of high levels of contaminating agents can affect the biological value of the colostrum or render it useless(18).

A possible physiological role played by microorganisms transferred from mother to baby during breastfeeding is not clearly explained in the literature. In this sense, the present study was carried out with the objective of obtaining data on the microbiota of the human colostrum. It is also our objective to correlate the microbiota with the possibility of being a source of probiotics, which would be transferred from mother to baby during natural breastfeeding.

Patients and methods

Seventy individual samples of colostrum were collected from the Mother-Infant Rooming-In Wards of the Instituto Fernandes Figueira from August 11th, 1999 to June 11, 2000 observing the use of asepsis techniques. Samples were collected by Human Milk Bank technical staff to stimulate lactation or to relieve mammary engorgement.

Out of the total, 24 (34.2%) donors were mothers of premature infants with gestational period varying from 36 to 37 incomplete weeks. Three out of these 24 mothers were being administered antibiotics at the moment of collection. Immediately after collection, the flasks of milk were transported using cold storage to the Human Milk Bank of the Instituto

Fernandes Figueira. At the Instituto, samples were collected and sent forth to the Laboratory of Food Control of the Instituto where the following analyses were carried out:

Mesophiles

Procedures were carried out according to methods described in the Compendium of Methods for the Microbiological Examination of Foods(19). 1.0-ml aliquots of colostrum and of selected decimal dilutions of colostrum were seeded in duplicate by pour plating in plate count agar (PCA; Merck). After solidification of the medium, plates were incubated at 35 degrees C for 48 hours. Colony counts were carried out and results expressed as colony forming units (CFU) per ml of colostrum.

Thermotolerants

The same procedures described for the mesophile were carried out. The criteria for classifying microorganisms as thermotolerants included those resistant to 63 degrees C for 30 minutes in tubes with 5 ml of colostrum and before inoculation into the culture medium(20); results were expressed as CFU per ml.

Psychrotrophs

The same procedures described for the mesophile were carried out with the exception of temperature and duration of incubation, which were of 7 degrees C for 10 days, respectively(20); results were expressed as CFU per ml.

Thermotolerant-Psychrotrophs

The same procedures described for the thermotolerants were carried out but with the same temperature and duration of incubation employed for the psychrotrophic(21); results were expressed as CFU per ml.

Proteolytics

Procedures were carried out according to Marth(22). 1.0-ml aliquots of selected dilutions were seeded in duplicate by pour plating in plate count agar (PCA; Merck) with 10% skim milk. After solidification of the medium, plates were incubated at 21 ± 2 degrees C for 72 hours. After incubation, plates were soaked in 3 ml of 10% acetic acid solution for one minute. Colony counts were carried out and results expressed as CFU per ml.

Proteolytic-Psychrotrophs

The same procedures described for the proteolytic were carried out with the exception of temperature and duration of incubation, which were of 7 degrees C for 10 days, respectively(21); results were expressed as CFU per ml.

Lipolytics

1.0-ml aliquots of selected decimal dilutions were seeded in duplicate by the plate count agar with tributyrin and incubated at 25 degrees C for 5 days(22). Colony counts were carried out and results expressed as CFU per ml.

Mold and yeast

Procedures were carried out according to Marvin(19). 1.0-ml aliquots of colostrum and selected decimal dilutions were seeded by pour plating in potato dextrose agar (PDA). After solidification of the medium, plates were incubated at 25 degrees C for 5 days. Colony counts were carried out and results expressed as CFU per ml.

Staphylococcus aureus

Procedures were carried out according to the Compendium of Methods for the Examination of Foods(19); 1.0-ml aliquots of colostrum and selected dilutions were seeded in duplicate and inocula were spread on the surface of Baird-Parker agar medium with a Drigalsky loop. Plates were incubated at 35 to 37 degrees C for 48 hours. Colony counts were carried out and results expressed as CFU per ml.

Total coliforms

Estimation of total coliforms was carried out based on the most probable number (MPN) according to the Standard Methods for the Examination of Dairy Products(22). Aliquots of selected decimal dilutions were inoculated in a three tube most probable number procedure with 2% brilliant-green-bile and lactose (Merck). Tubes were incubated at 37 degrees C for 24 to 48 hours. After incubation, we observed tubes for production of gas (positive). The MPN of coliforms was calculated with McGrady's table(19). The contents of gas-positive tubes were duplicated to new tubes with brilliant-green-bile and lactose using a bacteriological loop. These tubes were incubated at 37 degrees C for 24 to 48 hours for confirmation; results were expressed as MPN per ml.

Fecal Coliforms

Procedures were carried out according to the Microorganism in Foods compendium (23). Total-coliform-positive tubes were individually duplicated to new tubes using a bacteriological loop. The new tubes contained EC broth (Merck) and were incubated at 44.5 ± 0.1 degrees C through immersion in water bath for 24 to 48 hours. After incubation, we observed tubes for production of gas (positive); results were expressed as MPN per ml.

Group D Streptococci

Procedures were carried out according to the Laboratório Nacional de Referência Animal (National Laboratory of Animal Reference)(24). 1.0-ml aliquots of selected dilutions were inoculated in triplicate in tubes containing dextrose azide broth (Merck). These tubes were incubated at 36 degrees C for 48 hours; the MPN was calculated using McGrady's table.

Lactic bacteria

Procedures were carried out according to Lima(25) using PCA with supplementation of 0.004 g of bromocresol purple and 0.5 g of lactose per 100 ml of medium. In order to avoid diffusion of the acid produced by colonies in agar, we added 0.2% calcium carbonate. 1.0-ml aliquots of colostrum and selected decimal dilutions were seeded in duplicate by pour plating. Plates were incubated at 32 degrees C for 48 hours; colonies bound by a yellow halo were counted and results expressed as CFU per ml.

Results

Table 1 presents the percentage distribution of samples in relation to mesophiles (68.6%); thermotolerants (38.6%); psychrotrophs (8.6%); thermotolerant-psychrotrophs (0.0%); proteolytics (15.7%); proteolytics-psychrotrophs (1.4%); lipolytics (4.3%); mold and yeast (11.4%); *Staphylococcus aureus* (44.3%); total coliforms (7.2%); fecal coliforms (0.0%); Group D Streptococci (0.0%); and lactic bacteria (37.2%).

Table 1 -

Percentage distribution of microorganisms/groups in 70 colostrum samples

Discussion

The population of mesophiles was below 10^3 CFU per ml in 88.5% of samples analyzed. This rate was compatible with the prevalence of other microorganisms in this study. The group of mesophiles includes most of the contaminating agents, which allows for an overall idea of the total microbial load(26).

The presence of thermotolerants was observed in 38.6% of samples for a maximum count of 7.2×10^3 CFU/ml. This group, which is of secondary contaminating agents of the colostrum, is composed of microorganisms that resist to the pasteurization and heat treatment(21).

The occurrence of psychrotrophs was limited to six samples with counts below 4.3×10^2 CFU/ml. Almeida(21) posits that psychrotrophs in colostrum are secondary contaminating agents capable of growth during cold storage independently of their optimal growth temperature. The main strains of psychrotrophs include: *Enterobacter*, *Achromobacter*, *Flavobacterium*, *Alcaligenes*, *Pseudomonas*, *Bacillus*, and *Clostridium*. In addition, the last three strains are also included in the group of proteolytics(20).

The thermotolerant-psychrotrophs were absent in all samples analyzed. These are secondary contaminating agents with characteristics similar to those of the thermotolerants and psychrotrophs(27). The thermotolerant-psychrotrophs can resist to pasteurization and, thus, have a decisive effect on conservation of collected human colostrum(21).

The group of thermotolerant-psychrotrophs have the ability to grow even during cold storage and, thus, play an important role in the microbial ecology of the colostrum from the vantage point of conservation(21).

The presence of proteolytics was limited to 15.7% of samples with a maximum count of 1.4×10^3 CFU/ml. In turn, the proteolytic-psychrotrophic microorganisms (secondary contaminating agents combining characteristics of both proteolytics and psychrotrophs) were detected in 1.43% of samples. These latter microorganisms can promote proteolysis during growth in the colostrum at cold storage temperatures(27).

The presence of lipolytic bacteria was detected in three samples, with counts varying from 1.0×10^1 to 4.3×10^3 CFU/ml. These microorganisms can influence oxidative and hydrolytic lipid metabolism of the colostrum(28).

The presence of mold and yeast was registered in 11% of samples with counts of 1.3×10^1 to 6.0×10^3 CFU/ml. The presence of mold and yeast is associated with inadequate hygiene and sanitary conditions of donors(21).

Results indicated presence of *S. aureus* in 44.2% of samples with 100% of counts lower than 10^3 CFU/ml. The greatest concern as to the presence of these microorganisms is related to the appearance of enterotoxigenic *S. aureus*, which produces toxins resistant to pasteurization in population counts of 10^5 CFU/ml or higher(19).

The group of coliforms was detected in 5 samples with counts that varied from 0.3×10^0 to 1.1×10^2 MPN/ml. After submitting these samples to confirmation for total coliforms, results remained the same. In this sense, there were no fecal coliforms. According to the literature (19), the presence of fecal coliforms would indicate possibility of fecal contamination. The absence of group-D Streptococci confirms the results on fecal coliforms.

Populations of lactic bacteria were observed in 26 out of the 70 samples (37%), with all counts lower than 10^4 CFU/ml. Lactic acid bacteria (LAB) and the substances they produce exert beneficial effects in the gastrointestinal tract. LAB prevent adherence, establishment, and replication of several enteric mucosal pathogens through several antimicrobial mechanisms. LAB also release various enzymes into the intestinal lumen and exert potential synergistic effects on digestion(29).

During the last few years, the number of studies on probiotics has increased significantly with the discovery of several new strains of microorganisms, each presenting a variety of benefits. Reports of functional characteristics and benefits are common to several probiotics(30); bacteria that produce lactic acid are the most widely studied probiotics, especially the *Lactobacillus* sp. and the *Bifidobacterium* sp(2).

According to De Roos and Katan(15), the most widely-found probiotics in the recent literature are the *Lactobacillus* GG (22 studies), the *Lactobacillus acidophilus* (16 studies), the *Bifidobacterium bifidum* (6 studies), and the *Enterococcus faecium* (7 studies).

Clinical trials have shown that specific probiotic microbes, mainly lactic acid bacteria and bifidobacteria, can alleviate or prevent various intestinal disorders and reduce the risk for some intestinal diseases(31). The consumption of LAB-fermented products can present antitumoral effects. These effects are attributed to inhibition of mutagenic activity due to reduction of various enzymes involved in generation of carcinogenic and/or mutagenic substances(29).

Results from a qualitative and quantitative study with 26 different brands of yogurt indicated that isolate strains of *Lactobacillus* (considered probiotic) were found at concentrations of 10^5 CFU/g of the product before the expiration date(32).

The use of probiotic agents, mainly bifidobacteria, can exert an effect against acute diarrheas. In pediatric populations, the effect of probiotic agents appears to be most significant against viral (rotaviral) diarrhea, suggesting that an immunological mechanism is responsible for the beneficial effects(33). Others have also reported that oral administration of *Lactobacillus casei* strain Shirota (LcS) increases innate immunity, thus stimulating Natural Killer cell activity(5).

The *Lactobacillus casei* strain Shirota is one of the most widely used probiotic bacteria for production of fermented milk products and lactic acid bacteria preparations. A study has indicated fecal recovery of these bacteria of about 10^7 live bacteria per gram of feces, indicating that LcS survived transit through the gastrointestinal tract after ingestion of the

fermented milk(34).

According to Penna et al.(3), microorganisms can only exert influence on the ecosystem in which they are found with populations higher than 10^7 CFU per g or ml. In the specific case of intestinal flora of newborn infants, however, these values may be lower. Up to the present moment, we did not find in the literature reports of threshold values.

The pattern of bacterial colonization in the gut of premature infants is different from that of full-term infants. The earlier require intensive care, acquire intestinal organisms slowly, and their establishment of bifidobacterial flora is retarded(8). Delayed bacterial colonization of the gut with a limited number of bacterial species tends to be virulent because bacterial overgrowth is one of the major factors that promote bacterial translocation(8). Hence, feeding infants with colostrum from their mothers can present benefits in this aspect.

In principle, the samples obtained from mothers of premature babies who were using antibiotics did not present different results; however, since we did not establish controls for checking the influence of these parameters on the studied flora, it is not possible to assess these aspects.

The overall assessment of the results of our study indicated absence of pathogenic microorganisms and presence of secondary contaminating agents at levels incapable of compromising the microbiological quality of the colostrum. However, our results also indicate the presence of a significant microbiota, mainly of bacteria that produce lactic acid, which can probably operate as probiotics when made available to their receptors.

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